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by

A. A. Yabrov, S. A. Anatoly, O. V. Savitskaya, O. M. Bodazhkova, Z. M. Kruglikhina and M. I. Dudkina

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A. A. Yabrov, S. A. Anatoly, O. V. Savitskaya, O. M. Bodazhkova, Z. M. Kruglikhina, and M. I. Dudkina

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13. ABSTRACT

The results of testing the effect of bacterial toxins on tissue culture cells depended largely on the experimental conditions.

The cytopathogenic effect of the toxins under study is primarily determined by the degree of species, sensitivity of the cells. The results of these experiments may be also significantly influenced by the culture age, periods of changing the medium and time of recording the results.

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METHODICAL FEATURES ATTENDING THE WORK WITH BACTERIAL TOXINS ON TISSUE CULTURES

Nowadays all layers of culture cells receive much more and wider application in work with bacterial toxins (Penso and Vicari, 1957; Placido and Evans, 1957; Ujkelyi and Ormay, 1961; Shumakher with co-authors, 1963; P. H. Kicelev and G. E. Arkadeva, 1965; E. I. Belyanev, 1966; and others. Genuine works explains the influences and determined the conditions in setting up experiments to display a activity of some bacteriological toxin on cells of contaminated culture.

The more important tests utilized single layers of culture tissue in 11 samples of culture emb . The seed dose was $5 \cdot 10^5$ cells in 1 milliliter After the monolayer size was formed in an environment containing a serum of 5% hemohydrolysis and 5% of bull serum, supporting environment, which was serum free, was substituted.

Native diphtheria toxins were applied (the composition of toxicity for guinea pigs is 700 Dlm in 1 milliliter taphylococcus toxin (a hemolytic titration standard 1:512. Endotoxin S. Tuphi and Sh. flexneri acknowledged by the methods of Raistrika and Tonli (1934); 1 Dlm during

After toxins are placed in the test tubes with culture, the environment is changed, to definitely consider that the culture is conditioned by the cultivating fluids. For infectious cells utilization gradually raised the culture toxin. Everyday, contaminated material was detected in 3 test tubes.

The results took into account the present microscopic investigation of cultures. Microscopies are derived daily in the course of 7 to 10 days. Cytopatic action (TS P TS) toxin was manifested in forming granularity protoplasm, globose (spherical) cells and differently extended the complete breaking of the cellular layer. After the titer

toxin reached its limit of dilution, this method evoted a clearness of cytotoxin change in the culture.

Research showed what selection of culture tissue to work, which bacteria toxin to follow to differentiate its sensitivity to TS P D in relation to different forms of cells attachment. As illustrated in Table 1, cells of culture tissue from chicken embryos are considerably more sensitive to diphtheria toxin, than cells from a human embryo; its minimum active is compiled as corresponding to 1:6000 and 1:200 respectively. At the same time contamination of culture tissue of chicken embryo endotoxin Sh. flexneri cytopatic changed to illustrate that it does not turn over well, even if endotoxin is finally taken in a dilution of 1:2, then as in the culture of human cells, distinct cytopatic result is possible to receive during a 15 multiple of its dilution.

Tests of staphylococcus toxin on the same culture received the same results (see Table 1).

In all of the following tests the cells from the first layer of culture tissue from an 11 day old chicken embryo was used.

Table 1. Relative cytopatic Performance of toxin bacteria in cells from sensitive species of cultured tissue.

Toxin	Culture Tissue	
	Chicken	Human
Diphtheritic	6000[1]	200
Staphylococcal	25	20
Endotoxin Sh. flexneri	0[2]	15

[1]Figure in this and following tables denotes maximum detection of toxin, and illustrates clearly the power of cytopatic action.

[2]The dilution is characterized as <2.

Table 2. Relative cytopatic performance of toxin bacteria in cells from sensitive species of culture tissue.

Toxin	Days after introducing infectious culture		
	1st	2nd	3rd
Diphtheritic	2000	8000	12000
Staphylococcal	30	25	25
Endotoxin Sh. typhi	220	80	60

While studying the influences of the culture age on the sensitivity of cells to toxin changes in the environment produced on the 1st, 2nd and 3rd day after planting, and on the same day cells were infected. As seen in Table 2, expression TS P D with the one and same toxin was different depending upon the cultures age. Daily culture was less sensitive to diphtheritic toxin than that of 2-3 days; its titer - composition - is formed as 1:2000 and 1:8000 - 12000 respectively. At the same time that daily culture becomes more sensitive to action of endotoxin S. typhi (titer 1:220 and 1:80 - 1:60 respectively). Sensitiveness of cells culture difference in age is approximately equal to the action of staphylococcal.

During infection of the one and the same culture, climacteric substantial differences may be possible and noted in the environment, dependent upon the date of the environmental change. During later changes on the 2nd and 3rd day, as seen in Table 3, more cells show additional sensitivity to the workings of the diphtheritic toxin. Respective titers therein exceeded 3 times more than the titer received during the change in the environment, in 24 hours after sowing. During infection staphylococcal daily changed the environment in culture cells yet did not render any essential influence on the results.

The introduction of endotoxin S. typhi, in culture during the 1st and 2nd day after the initial sowing, had the power to depress cytotoxinical action on the changed environment. So, after infecting the culture daily for three days, the clearness of cytotoxinical effect on changing the environment and the results - appeared during 30 multiple dilution endotoxin; then as during its introduction over the 1st or 2nd day after changes in the environment TS P D was uncovered, if only during the least dilution of endotoxin without its full capacity (see Table 3).

To study this phenomenon reading in more detail, one must combine the accumulation of paracells in a liquid substance, inhibiting TS P D endotoxin. If in the daily culture, sensitivity increased endotoxin action, instead of introducing a culture liquid in a supporting environment, i.e., likewise with environment No. 199. Sampled after its 2nd or 3rd daily contact with normal culture cells, TS P D toxin is sharply weakened. As seen in Table 4, that immediately afterwards, infection with S. typni (of the 1st, and 3rd days culture, the cells change their environment and the development pronounced sensitiveness to its action. Titer toxin was equalized at 1:180 and 1:50 respectively. In the presence of a tissular like liquid, and two days of preliminary contact, the environment receives supporting cells and the effect of endotoxin depressed pressure on the culture tissue was titer 1:4 and 0

respectively. Following this recording, TS P D diphtheritic and staphylococcal toxin tested on the culture liquid did not exert any pressure.

Table 3. Relative cytopatic performance of toxin bacterial during the period of the changing environment in infectionability tissue culture.

Toxin	Daily change in 3 days of infectionability culture		
	1st	2nd	3rd
Diphtheritic	3500	9000	10,000
Staphylococcal	30	35	30
Endotoxin S. typhi	0	4	30

Minimum term of calculating TS P D for separating toxin differences is presented in Table 5. First cleared change under influences associated with titer dilution diphtheria toxin was not recorded earlier than 2 days after the infection. Additionally they were formed to reach a definite limit from 5-7 days. Cytopatic active endotoxin S. typhi and staphylococcal were clearly pronounced already on the day following active infection. Changes excited the endotoxin, to the extent that 3-4 days growth was formed.

During studies of cytopatic staphylococcal toxin in dynamics it may be possible to especially note that definate TS P D was pronounced on the day following the infection. However, during the 2nd twenty-four hours after the introduction of toxin cytopatic formations cease to result. Deposits and recovery changes were observed in cells. Additionally, clearness returned to the changes expressed in the test tube, where the least damaging dose of toxin was introduced. Here infection was less pronounced. On the 2nd and 3rd day, they disappeared. It was evident from visual inspection that active toxin did not cease on the 4th day as symptoms of damage appeared anew. Additionally, harmful active damage increased.

Table 4. Relative cytopatic performance endotoxin of S. typhi paracells in a cultural liquid environment.

Cultured Tissues	Supporting environment utilization before infectionability	
	Environment No. 199	Cultural Liquid[1]
Daily Variation	180	4
3 Days Variation	50	0

[1]The cultured liquid received culture cells from 3 daily normal environments over 2 days after change.

Table 5. Dynamics cytopatic changes producible effectiveness of bacterial toxin on cells from culture tissues of embro.

Toxin	Days after infection				
	1st	2nd	3rd	4th	7th
Diphtheritic	50	1500	2500	3000	5000
Staphylococcal	30	20	20	25	35
Endotoxin S. typhi	80	100	110	110	110

So in the specimen, cytopatic calculation changes of producible diphtheria toxin was not produced earlier than in the 2nd twenty-four hours after infection. In calculating the changes, producible effectiveness of endotoxin S. typhi, it is possible to note it during the 2nd day.

Results of the active staphylotoxin follows on the first day, after introducing toxin into the culture. During the following days given, and in the one and same tests, the results were considerable different. Following was noted; only in a week after infection it was possible to prove the unauthenticity of the calculated results in all cases. So stratification is more probable in longer cultivation as non-specific changes combine with aging culture.

Conclusions

1. Study of cytopatic active diphteria and stapylococcal toxin, and also endotoxin stimulant of intestines groups in culture cells of chickens and humans of embryos show, that results were tested in considerable degree and the understanding thereof depend upon the established tests. So, the study expressed that cytopatic active toxin depend not only on the species sensitivity of the cells to the employed culture, its age, the date of the environment, but also the time of following-up the experiment.

2. Results of the research presented evidence for the necessity of very strickly selecting and observing the conditions during tests of cytopatic active differences in bacterial toxin.

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